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Stripping voltammetric and polarographic techniques for the determination of anti-fungal ketoconazole on the mercury electrode

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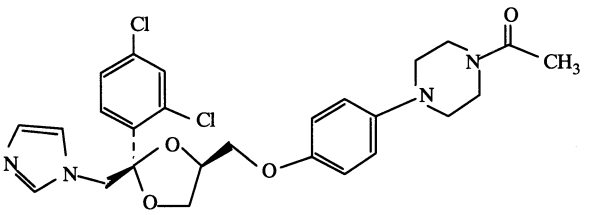
Abstract

The electroanalytical behaviour of ketoconazole in Britton–Robinson buffer is described. The reduction process on the hanging mercury drop electrode (HMDE) gives rise to one peak over −1.6 V (vs. Ag/AgCl/sat.KCl), within the pH range studied (4.7–9.6). The results showed that the reduction of ketoconazole is irreversible and the limiting current is adsorption controlled. The dependence of the peak current on the concentration was studied by means of different polarographic and voltammetric techniques. Using adsorptive stripping differential pulse voltammetry (AdS-DPV), the detection limit (DL) reached was 5.3×10^{-11} mol l^{-1}. Two procedures, based on differential pulse polarography (DPP) and AdS-DPV in aqueous medium were developed for the determination of ketoconazole in a gel formulation and spiked urine samples, respectively.

Keywords: Ketoconazole; Polarography; Cathodic stripping voltammetry; Anti-fungal

1. Introduction

Ketoconazole is an imidazolic derivative which is commonly used as an anti-fungal drug to treat superficial and systemic mycoses [1] and whose general structure is:



Ketoconazole is metabolised by the hepatic microsomal oxidation system, and only a small fraction (< 1%) of the administered dose (200–400 mg) was excreted unchanged in the urine over 6 h [2]; for this reason, a highly sensitive analytical

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method is essential for the evaluation of this drug in urine.

Various analytical methods have been developed for the determination of ketoconazole, among them some including VIS spectrophotometry [3–6], UV spectrophotometry [7–9], spectrofluorimetry [10], thin-layer chromatography [11], supercritical fluid chromatography with UV detection [12] and capillary electrophoresis with diode array detection [13,14]. But up to now, the most sensitive and commonly employed methods for the determination of this drug have been based on liquid chromatography using different detection modes such as UV spectrophotometry [15–30], diode array detector [31] and electrochemical detection [32]. The presence in the ketoconazole molecule of electro active groups makes it an interesting candidate for electroanalytical methods for analysis. The earlier paper, related to polarographic investigation of ketoconazole by applying a oscillo-polarographic method in an aqueous 20% ethanol phosphate buffer (pH 7.45), was described by Fijalek et al. [33] reaching a DL close to 1 ppm. The more extensive electrochemical studies of ketoconazole oxidative properties have been done by Shamsipur and co-workers, in a chloroform solution on different class of electrodes (Au, Pt and GC) [34] and in Britton–Robinson buffer (pH 12) using a bare carbon paste electrode [35]. Recently, Peng [36] reported the development of a more sensitive anodic stripping voltammetric method on the glassy carbon electrode in NH_3–NH_4Cl buffer (pH 9). These electroanalytical procedures, some of them based on the oxidation of ketoconazole, offer a poor sensitivity and they are performed in aqueous organic mediums. There are no voltammetric methods in the literature for the determination of ketoconazole on the mercury electrode. Besides, this drug had strong adsorptive properties. The aim of the present work is to study the electrochemical reduction of ketoconazole in aqueous medium (Britton–Robinson buffer) on the mercury electrode, using several modern polarographic and voltammetric techniques. It is also aimed here to develop sensitive electroanalytical procedures for the direct determination of ketoconazole at two different concentration levels, in a gel formulation and spiked urine samples using differential pulse polarography (DPP) and adsorptive stripping differential pulse voltammetry (AdS-DPV), respectively. The proposed methods avoid the use of organic solvents, which present high volatility and toxicity, and are simple, sensitive, rapid, reproducible and easy to apply in routine determinations. The resulting accuracy and precision are at least comparable, or even better, to the methods described above by other authors.

2. Experimental

2.1. Apparatus

All polarographic and voltammetric curves were obtained using a PGStat 10 and a PGStat 20 voltammetric analysers from Eco Chemie (Utrecht, Netherlands) and GPES 4 software (Eco Chemie). The voltammetric system was linked to a Metrohm 663 VA stand (Herisau, Switzerland), which had a multimode electrode (MME) in the hanging mercury drop (HMDE) mode as working electrode, with a mean drop size of 0.52 mm^2. An Ag/AgCl/sat.KCl reference electrode and a platinum auxiliary electrode completed the three-electrode system. A stirring bar provided convective transport during the preconcentration step, and a Metrohm E-605 pHmeter was used to measure pH values. A Beckman P/ACE 5510 CE system provided with a diode-array detection system and Beckman software station 1.0 was used (Beckman instruments, Fullerton, CA, USA). CZE was carried out in an uncoated fused-silica capillary tubing (40 cm effective length $\times$ 75 μm I.D.). All measurements were performed at a temperature of 20 ± 0.5 °C.

2.2. Reagents

All solutions were prepared from analytical-grade chemicals with de-ionised water from a Milli Q system (Millipore, Bedford, MA, USA) except ketoconazole, which was provided by Acufarma (Tarrasa, Spain). C-8 solid-phase extraction cartridges (1 ml) (Supelco, Bellefonte, PA, USA) were used. A 0.04 mol l^{-1} Britton–Robinson buffer solution was used as the supporting electrolyte.

The pH was adjusted with a NaOH solution (0.2 mol l^{-1}). An aqueous 1.0×10^{-3} mol l^{-1} ketoconazole stock solution was prepared by dissolving the pure product *cis*-1-acetyl-4-[4-[[2-(2,4-dichorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]-methoxy]phenyl] piperazine (F.W. 531.44) in Britton–Robinson solution. Working standard solutions were obtained by suitable dilution in B–R buffer and stored at 4 °C. In these conditions, the ketoconazole solutions are stable and its concentration does not change with time. The pharmaceutical dosage form used was Fungarest gel (Janssen-Cilag, S.A., Madrid). Each ml contains: ketoconazole 20 mg, and sodium lauryl sulphate, disodium monolauryl sulfosuccinate, diethanolamine, imidurea, erythrosine and HCl, as excipients.

2.3. Analysis of gel formulation samples

Fungarest gel was heated in a boiling water bath, and a solution was prepared by diluting the commercial drug form with supporting electrolyte to 1:1000 v/v. Then, 50 μl of this solution were placed in a polarographic cell, which contained an aliquot of 25 ml of buffer solution (Britton–Robinson, pH 5.6). The solution was deoxygenated for 10 min with a stream of pure nitrogen and then maintained in a nitrogen atmosphere. The determination by square wave (SWP) (Osteryoung's method) or by DPP was carried out with the optimised instrumental parameters shown in Table 1. The potential range scanned was from 0 to −2000 mV. Measurements were carried out at 20 ± 0.5 °C and the ionic strength was adjusted to 0.2 mol l^{-1} $NaClO_4$. The standard addition method was then applied adding successive aliquots of 100 μl of a 1.0×10^{-5} mol l^{-1} ketoconazole solution for the determination of drug concentration.

2.4. Analysis of urine samples

Urine was spiked at 6.25×10^{-8} mol l^{-1} ketoconazole level. A sep-pack cartridge was conditioned by activating the C-8 resin with two successive additions of pure methanol (2.0 ml) and Britton–Robinson buffer (2.0 ml of 0.04 mol l^{-1}) at pH 5.6. Then, 1.0 ml of urine spiked was applied. To remove urine impurities from the cartridge, two successive washings with 1.0 ml of methanol–water solution (40:60, v/v) and 1.0 ml of acetone–water solution (20:80, v/v), were carried out. Finally, ketoconazole was eluted with 1 ml of pure methanol. 200 μl of this sample were added to the voltammetric cell, which contained 25 ml of supporting electrolyte (B–R buffer) at pH 5.6. Nitrogen was bubbled through the solution for 10 min to deareate it. On the deposition step at −1.4 V, the solution was stirred at 2000 rpm for 80 s. After a 5 s rest time, a cathodic potential sweep was carried out from 0 to −2000 mV using SWV or DPV as redissolution techniques with its optimum instrumental parameters (Table 1). Measurements were made at 20 ± 0.5 °C and the ionic strength was adjusted to 0.2 mol l^{-1} $NaClO_4$. Quantification was performed by means of the standard addition method, adding successive aliquots (200 μl) of 1.0×10^{-7} mol l^{-1} ketoconazole solution.

3. Results and discussion

3.1. Characteristics of the electrode process

Ketoconazole exhibits one reduction peak close to −1.8 V, at pH 5.6 on the HMDE, as can be observed in cyclic voltammograms, in Fig. 1a, without preconcentration, and after a preconcentration step (−1.4 V, 80 s, 2000 rpm) (Fig. 1b). The peaks obtained without accumulation were substantially smaller than those obtained after a preconcentration step; thus indicates that ketoconazole adsorbs readily at the electrode surface, and a considerable increase in sensitivity can be gained by adsorptive accumulation prior to the voltammetric determination. The cathodic wave is not accompanied by a corresponding anodic one, which indicates the non-reversibility of this process.

The influence of pH on the $E_{1/2}$ and the limiting current for the reduction peak, using direct current polarography (DCP), has been studied. The potential shifted linearly to less negative values with increasing pH, following the equation: $E_{1/2} = -$

Table 1
Optimum operational parameters selected for the determination of ketoconazole by polarographic and voltammetric techniques on the mercury electrode

Parameters	Variation interval		Optimum values	
			DP	SW
Step (mV)	1	20	10	1.5
Pulse amplitude (mV)	20	100	100	80
Modulation time (ms)	2	100	3	—
Interval time (s)	0.1	1.0	0.2	—
Frequency (Hz)	10	250	—	150
PH	4.7	9.6	5.6	5.6
Ionic strength (M) $NaClO_4$	0.01	0.40	0.2	0.2
Deposition time (s)*	10	250	90/180	90/180
Deposition potential (V)	0	−1.6	−1.4	−1.4
Stirring speed (rpm)	0	3000	2000	2000
Rest time (s)	0	30	5	2000

*, At two concentration levels: 2.0×10^{-8} and 2.0×10^{-10} mol l^{-1}.

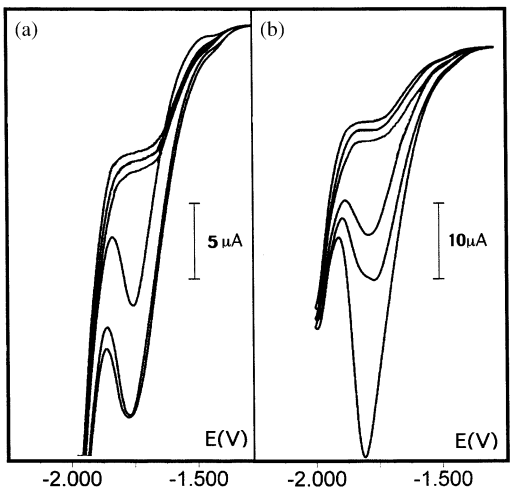

Fig. 1. Cathodic–anodic repetitive cyclic voltammograms for a 1.6×10^{-7} mol l^{-1} ketoconazole solution in Britton–Robinson buffer (pH 5.6) using a scan rate of 100 mV s^{-1}: (a) without accumulation; (b) after a preconcentration step, tacc 80 s, Eacc −1.4 V and stirring speed 2000 rpm.

2.02 V+0.07 pH. This indicates that a transfer of protons is coupled to the reduction process. Regarding the limiting current value, it increases slightly when pH rose up to a value of 5.6, where a maximum was observed, and this value was selected as optimum for the quantitative determination of ketoconazole. These polarographic and voltammetric results in weakly acidic media revealed a good agreement with the electrochemical behaviour described for similar amide compounds [37] suggesting that the cathodic peak of ketoconazole could be due to the irreversible reduction of carbonyl group.

3.2. Optimisation of analytical conditions

Taking into account that the DP and SW techniques were used to develop methods for quantitative determination of ketoconazole, and in order to choose the optimum conditions, some instrumental variables were studied on a 4.0×10^{-7} mol l^{-1} ketoconazole solution, and the optimised values are exposed in Table 1.

The influence of pH and ionic strength on the reduction peak height were studied by means of SWP on a 1.0×10^{-7} mol l^{-1} ketoconazole solution. In our working conditions, the concentration of $NaClO_4$ background solution affected slightly the analytical signal, and the ionic strength was adjusted to 0.2 M $NaClO_4$. The largest peak current was obtained at pH 5.6, as expected.

The influence of time on the stability of solutions was also checked for a week and no significant variations were observed on the drug concentration. Sometimes the influence of light results in a change in the red-ox properties of the

compound, and the extent of the photodegradation can be followed electrochemically via a decrease in the reduction peak current or by the appearance of new reduction waves at a more positive potentials. Ketoconazol seems to be less light sensitive, as no degradation could be observed after exposure to daylight for 6 h. The temperature coefficients $[\tau = 1/(T_2 - T_1) \cdot \ln i_2/i_1]$ calculated in the range 20–51 °C were: 1.85% $°C^{-1}$ (20–27 °C), 1.68% $°C^{-1}$ (27–31 °C), 1.39% $°C^{-1}$ (31–35 °C), 1.21% $°C^{-1}$ (35–39 °C), 1.12% $°C^{-1}$ (39–43 °C), 1.10% $°C^{-1}$ (43–47 °C) and −1.01% $°C^{-1}$ (47–51 °C) respectively. Thus, except for temperature, no special precautions are necessary during the voltammetric measurements and storage. Therefore, the polarographic method proposed can be used as a stability-indicating method.

3.3. Influence of various parameters on adsorptive stripping voltammetry

Once the most suitable instrumental and solution conditions for redissolution techniques were established, it was necessary to select the variables affecting the adsorptive process; for this purpose, SWV as redissolution technique and a 2.0×10^{-8} mol l^{-1} ketoconazole solution were used. The results are exposed in Table 1.

The application of different accumulation potentials, between 0.1 and −1.6 V, concluded that the maximum value for the peak current occurred at −1.4 V. The peak current increased steadily when the stirring speed varied between 0 and 3000 rpm and a value of 2000 was selected as optimum. The effect of accumulation time on peak current showed that the optimum preconcentration time before the electrode became saturated was 90 s (2.0×10^{-8} mol l^{-1}) and 180 s (2.0×10^{-10} mol l^{-1}). The rest time was varied up to 20 s, and a value of 5 s was chosen as optimum, which allows the adsorbed substance to homogenise on the electrode substance.

3.4. Influence of concentration on peak current

Using the optimum conditions mentioned above (Table 1), the quantitative determination of ketoconazole was carried out, based on the linear dependence of the peak current on concentration. Table 2 shows the results of regression data of calibration lines (L.R., linear range; b, slope; a, intercept, S_b, standard deviation of the slope, S_a, standard deviation of the intercept; S_{yx}, error standard deviation; r, correlation coefficient; n, data number; D.L., detection limit.) obtained using polarographic (DPP and SWP) and voltammetric techniques (AdS-DPV and AdS-SWV).

For DPP, a wider linear range than for SWP was observed (Table 2) and the DLs defined as $a + 3S_{yx}/b$ [38] were almost the same 7.1×10^{-8} mol l^{-1} (0.04 ppm) and 7.5×10^{-8} mol l^{-1}, respectively. The reproducibility of these methods were determined by successive measurements of ten ketoconazole solutions of 5.0×10^{-7} mol l^{-1} with variation coefficients (VC) of 2.1% (DPP) and 2.7% (SWP).

When stripping techniques were used, the plot I_p versus concentration levelled off at higher concentrations, as expected for a process that is limited by adsorption of analyte.

For quantitative determination using AdS-DPV one linear range was obtained between 1.0×10^{-11} and 1.0×10^{-9} mol l^{-1}, while for AdS-SWV a more narrow linear calibration plot was obtained in the range 2.0×10^{-10}–1.0×10^{-8} mol l^{-1} (Table 2). The DLs reached were 5.3×10^{-11} mol l^{-1} (0.03 ppb) and 1.7×10^{-10} mol l^{-1} respectively, and the VCs for a 6.0×10^{-10} mol l^{-1} ketoconazole solution (n = 10) were 1.9% for AdS-DPV and 2.8% for AdS-SWV.

The results in Table 2 show that AdS-DPV is more suitable than AdS-SWV since the DL and the VC are lower and the slope of the calibration line is higher (slope ratio 11.6). For polarography, both DPP and SWP methods present quite the same sensitivity, DLs and VCs; only the linear calibration range is wider for DPP and, therefore, more appropriate than SWP. Robustness [39] was examined by evaluating the influence of small variation of some of the most important procedure variables including E_{acc}, t_{acc}, pH, temperature, ionic strength, frequency (Square wave), pulse amplitude and step. The results showed that none of these variables significantly affects the determination of ketoconazole. Related to rugged-

Table 2
Regression data for calibration lines for the determination of ketoconazole by SWP, DPP, AdS-SWV and AdS-DPV on the HMDE

Parameter	SWP	DPP	AdS-SWV	AdS-DPV
LR (mol l^{-1})	$(1–20) \times 10^{-7}$	$(1–100) \times 10^{-8}$	$(2–100) \times 10^{-10}$	$(1–100) \times 10^{-11}$
b (μA l mol^{-1})	3.1×10^{7}	2.1×10^{7}	1.2×10^{8}	13.9×10^{8}
a (μA)	2.29	0.93	0.03	0.62
S_b (μA l mol^{-1})	4.6×10^{5}	5.6×10^{5}	5.0×10^{5}	3.2×10^{7}
S_a (μA)	0.39	0.25	2.3×10^{-3}	1.8×10^{-2}
S_{yx} (μA l mol^{-1})	0.77	0.49	6.9×10^{-3}	2.5×10^{-2}
r	0.9990	0.9990	0.9999	0.9982
n	11	14	18	12
DL (mol l^{-1})	7.5×10^{-8}	7.1×10^{-8}	1.7×10^{-10}	5.3×10^{-11}

Optimum conditions in Table 1. L.R., linear range; b, slope; a, intercept, S_b, standard deviation of the slope, S_a, standard deviation of the intercept; S_{yx}, error standard deviation; r, correlation coefficient; n, data number; D.L., detection limit.

ness, the results obtained due to day to day and even lab to lab variations were found reproducible since there was no significant difference between the recovery results, when the developed procedures to determine ketoconazole were carried out using two different voltammetric analysers (PGSTAT 10 and PGSTAT 20) with the optimised instrumental parameters shown in Table 1.

3.5. Ketoconazole assay in gel formulation samples

Fungarest gel contains as main excipients two surfactants (sodium lauryl sulphate and disodium monolauryl succinate), and as it is well known, the presence of cationic, anionic and non-ionic surfactants at an adequate concentration level can all increase the peak current of the drug in polarographic determinations, being Triton X-100 the most commonly used sensitiser. In our working conditions, the adequate dilution of Fungarest gel sample with supporting electrolyte (1:1000 v/v) favours the determination of its ketoconazole content owing to the presence of these two surfactants. Neither was there any interference after dilution from the other excipients: diethanolamine, imidurea, erythrosine and HCl. Consequently, no sample pre-treatment is required different from dilution (1:1000 v/v) with the B–R buffer. After a single heating step and the adequate dilution of analyte present in Fungarest gel, five aliquots of this solution were analysed by DPP using the standard addition method (Fig. 2A), and the corresponding mean value was 19.68 mg ml^{-1} (recovery 98.42%, VC 2.5%), which is in accordance with the drug amount declared in Fungarest gel formulation (20 mg ml^{-1}). This method was compared with an independent method [14] (Capillary zone electrophoresis with photodiode array detection at 196 nm). The recovery percentage was 99.58% with a VC of 1.26%. As it can be observed there is a good agreement between the results

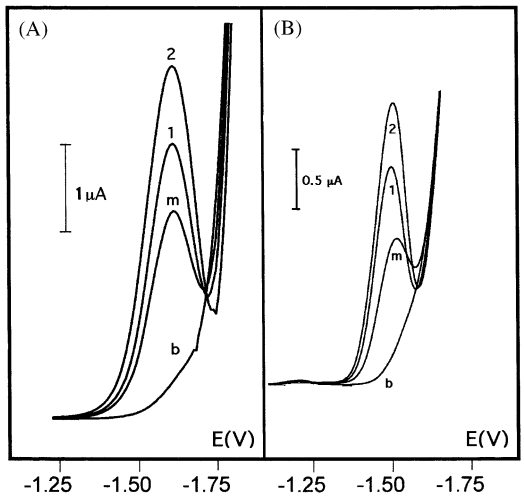

Fig. 2. DPP and AdS-DPV curves obtained for the determination of ketoconazole. (A) Fungarest™ gel: (b) blank, (m) 50 μl of gel sample; 1 and 2 standard additions of 100 μl of 1.0×10^{-5} mol l^{-1} ketoconazole solution. (B) Spiked urine (at 6.25×10^{-8} mol l^{-1} ketoconazole level): (b) blank, (m) 200 μl of urine sample; 1 and 2 standard additions of 200 μl of 1.0×10^{-7} mol l^{-1} ketoconazole solution. For other conditions, see Table 1.

obtained with both methods; and the application of Student's t-test and F-test indicate that the method is accurate and precise [38].

3.6. Ketoconazole assay in spiked urine samples

The effect of endogenous compounds of urine on the electrode response was considered by injecting amounts of 50, 100, 200, 500 and 1000 μl of urine into the electrochemical cell containing B–R buffer (pH 5.6) and a ketoconazole standard of 1.00×10^{-7} mol l^{-1}. Results showed that the peak current decreased as the concentration of urine in the cell increased. For this reason, direct determination of Ketoconazole in urine is not possible and a separation procedure based on a solid-phase extraction in C-8 cartridges was, therefore, necessary to minimise interferences. The cartridge was first conditioned with two successive additions of pure methanol (2.0 ml) and Britton–Robinson buffer (2.0 ml of 0.04 mol l^{-1}) at pH 5.6. Then, 1.0 ml of urine spiked was applied. To remove urine impurities from the cartridge, two successive washings with 1.0 ml of methanol–water solution (40:60, v/v) and 1.0 ml of acetone–water solution (20:80, v/v), were carried out. Finally, ketoconazole was eluted with 1 ml of pure methanol.

Fig. 2B shows the determination of ketoconazol in spiked urine at a concentration level of 6.25×10^{-8} mol l^{-1}, by means of AdS-DPV using the standard addition method. The mean recovery for the determinations carried out in five eluted urine aliquots of 200 μl was 100.81%, with a VC of 3.7%. As an amount of 4 mg ketoconazole is excreted unchanged in the urine over 6 h after the dose administration [2], the method is sensitive enough to determine ketoconazole in urine. It should be noted that, other authors [34–36] also consider voltammetry based on oxidation of ketoconazole molecule as very suitable methods to determine this drug in urine samples. Sensitivity may be enhanced by increasing the size of urine sample used, or by reducing the volume in which the extracted urine could be reconstituted after elution. Selectivity is sufficient for urine samples after the separation procedure.

4. Conclusions

A study of the reduction of ketoconazole in aqueous medium (pH 5.6) has been carried out. In our working conditions, the process is irreversible and mainly controlled by adsorption. Two electroanalytical methods based on DPP and AdS-DPV have been developed. Using the DPP method (DL 0.04 ppm), sensitivity has been improved with respect to other proposed methods based on: oscillo-polarography (DL 1 ppm) [33], voltammetry (DL 50 ppm) [34], UV–Vis spectrophotometry (DL 1–50 ppm) [3–9], fluorimetry (DL 0.2 ppm) [10] and capillary electrophoresis (DL 0.3 ppm) [13,14].

The DL for ketoconazole determination in urine, using AdS-DPV (DL 0.03 ppb), has been improved in three magnitude orders with respect to typical HPLC procedures described (DL 10–200 ppb) [12,15–32] and AdS-DPV (DL 12.4 ppb) [35]. It is comparable to the AdS-DPV results reported by Peng (DL 0.03 ppb) on the glassy carbon electrode, although usually oxidative voltammetric procedures on a glassy carbon electrode, compared with reductive measurements at the HMDE, proved to be less sensitive [34,35] and much more care had to be taken as to surface renewal and reproducibility of the individual measurements.

The present results show that AdSV at the HMDE is a very powerful technique for the determination of ketoconazole in low concentrations even in biological matrices. The sensitivity is significantly enhanced by adsorption of the drug on the electrode surface and, after careful choice of the operating parameters, extremely low DLs can be reached. Compared with other techniques the method is cheap and the measurements is not time consuming, leading to results for analytical purposes of this drug. That is adequately accurate and precise. The proposed methods avoid the use of organic solvents, which present high volatility and toxicity.

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